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Article | DOI: 10.21307/jofnem-2019-076

JOURNAL OF NEMATOLOGY

e2019-76 | Vol. 51

The morphological characteristics and phylogenetic analysis of *Pratylenchus vulnus* Taiwan strawberry isolate

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This paper was edited by Zafar Ahmad Handoo.

Received for publication June 24, 2019.

Abstract

Pratylenchus vulnus was discovered in nematode-distributing fields from symptomatic seedling roots and corresponding rhizosphere soil on strawberry farms in Taiwan. Microscopic measurements and scanning electron microscope observations of both sexes of the nematode coincided with the general morphological descriptions of the species. Four different types of female tail termini were observed, including pointed, digitate, smooth and tapering. Molecular analysis of the ribosomal RNA sequence (SSU, ITS and LSU regions) and the mitochondria COI gene sequences confirmed the species identification. Phylogenetic analysis suggested no specific geographic linkage of the Taiwan population to other previously reported populations.

Keywords

Pratylenchus vulnus, Morphology, SEM, Phylogenetic analysis, Strawberry, Root lesion nematode.

Root lesion nematodes (*Pratylenchus* sp.) are among the most economically damaging phytoparasitic nematodes on fruits, tree rootstocks and vegetables (Yu et al., 2012). *Pratylenchus vulnus* was first reported in 1951 as a pathogen of multiple trees and vines in California, USA (Allen and Jensen, 1951) and was later found to infect over 80 plant species (Castillo and Vovlas, 2007). In Uruguay, Sri Lanka and Australia, serious damage in strawberry (*Fragaria* × *ananassa*) fields caused by this nematode have been reported (Colbran, 1974; Minagawa and Maeso-Tozzi, 1990; Mohotti et al., 1997). We here report the discovery of one *Pratylenchus* sp. population in strawberry fields in Dahu township of Miaoli, Taiwan in 2017. The strawberry crops growing in the nematode-distributing fields GS and KDL were obviously stunted when discovered. Both fields had been cropping strawberry for over 10 years. The soil composition of the area was characterized as sandy loam by hydrometer method (Bouyoucos, 1951). Nematodes were extracted from 100 g ± 5% soil with modified Baermann funnel technique (Hooper, 1986). Morphological observations and measurements of adults (30 females and 30 males) were conducted with a compound microscope at magnification of up to 1000× (Table 2). The dorsal gland orifice of the female of our isolates fit the description of *P. vulnus* perfectly, with a range between 3.0 and 4.42 (means = 3.55), rather than 1.9 to 3.0 characteristic of *P. penetrans* (Roman and Hirschmann, 1969). All nematodes observed had a lateral field composed of four incisures with wider inner band but two types of lateral field were observed. The first type had an evenly uplifted lateral field (Fig. 1E), while the second type carried uplifted and thinner outer bands (Fig. 1F). Four different types of female tail termini were observed, including pointed, digitate,

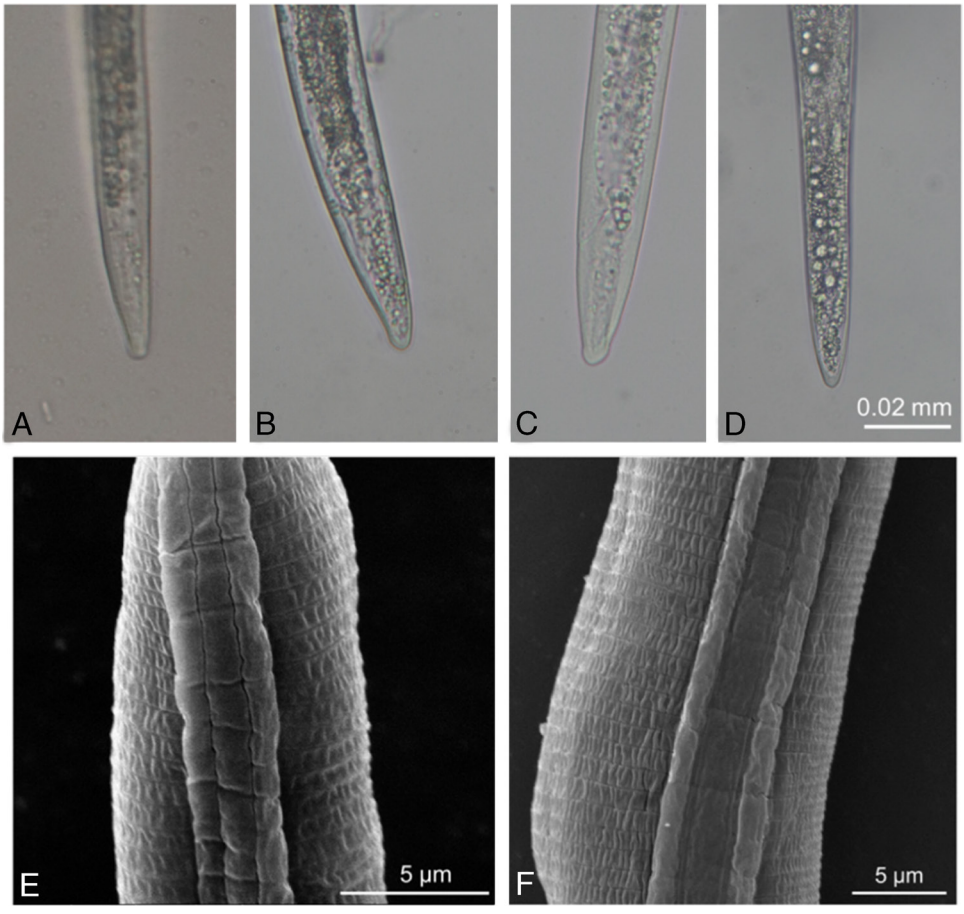

Figure 1: The morphological variations within the *P. vulnus* Taiwan strawberry population. Two lateral field types and four tail termini were observed. Photographs were taken at 1000X magnification with compound microscope and SEM.

smooth and tapering (Fig. 1A-D). *P. vulnus* is known to express morphological differences when cultured under different temperature conditions (Doucet et al., 2001). A previous report also characterized five *P. vulnus* groups in Japan by tail morphology (Mizukubo 1990). The fact that four different tail terminus types were observed in the *P. vulnus* Taiwan population implies this population may have been established in the region rather than being recently imported.

Molecular analysis of the ribosomal RNA and mitochondrial gene sequences of the extracted nematodes confirmed their species as *P. vulnus* (Table 1). The rDNA LSU region (D2A/D3B: ACAAGTA CCGTGAGGGAAAGTTG/TCGGAAGGAACCAGCT ACTA) (Nunn, 1992), rDNA ITS region (TW81/AB28: GTTTCCGTAGGTGAACCTGC/ATATGCTTAAGTTCA GCGGGT) (Amiri et al., 2002; Subbotin et al., 2001), rDNA SSU region (SSU18A/SSU26R: AAAGATTAAG CCATGCATG/CATTCTTGGCAAATGCTTTCG) (Eyualem and Blaxter, 2003) and mtDNA COI region (JB3/JB4.5: TTTTTTGGGCATCCTGAGGTTTAT/TAAA GAAAGAACATAATGAAAATG) (Derycke et al., 2010) of the Pv-GS and Pv-KDL are 99.04, 95.96, 99.66 and 99.24% identical, respectively. Both Pv-GS and Pv-KDL isolates had maximum 100% similarity of their rDNA LSU sequences to *P. vulnus* (GenBank accession: U47547.1). The Pv-KDL mtDNA COI region sequences of isolates were 99.74 to 100% similar to other sequences of *P. vulnus* available in the database (GenBank accessions KY424094, KY424095, KY828312, KY828317, KY424096-7 and KX349427), and only 81.08% identical to the second most closely related species, *P. scribneri* (GenBank accession: KY424089.1). The rDNA SSU region sequence analysis provided a similar result as Pv-KDL and are 99.42% (GenBank accession: KY424163) to 99.88% (GenBank accession: KY424164) identical to all available *P. vulnus* sequences in GenBank, and only 94.48%

Table 1. GenBank sequence deposit information of multiple gene regions of *P. vulnus* Taiwan isolates from strawberry fields GS and KDL.

P. vulnus sequences GenBank deposit information				
		rDNA		mtDNA
Isolate location	LSU (730 bp)	ITS (669 bp)	SSU (883 bp)	COI (441 bp)
GS	MG372808	MG372806.1	MG372807	MN431203
KDL	MK713641	MK713613	MK713614	MK764689

similar to *P. kumamotoensis* (GenBank accession: AB905295.1). The rDNA ITS sequences also separated the Pv-KDL from *P. kumamotoensis* (GenBank accession: KT175521.1) clearly with a very low 86.22% similarity. Phylogenetic analysis of the rDNA ITS and LSU regions combined suggested the nematodes from the two fields belong to one population (Fig. 2) and are isolated from the rest of the previous populations from other geographic regions (Table 2).

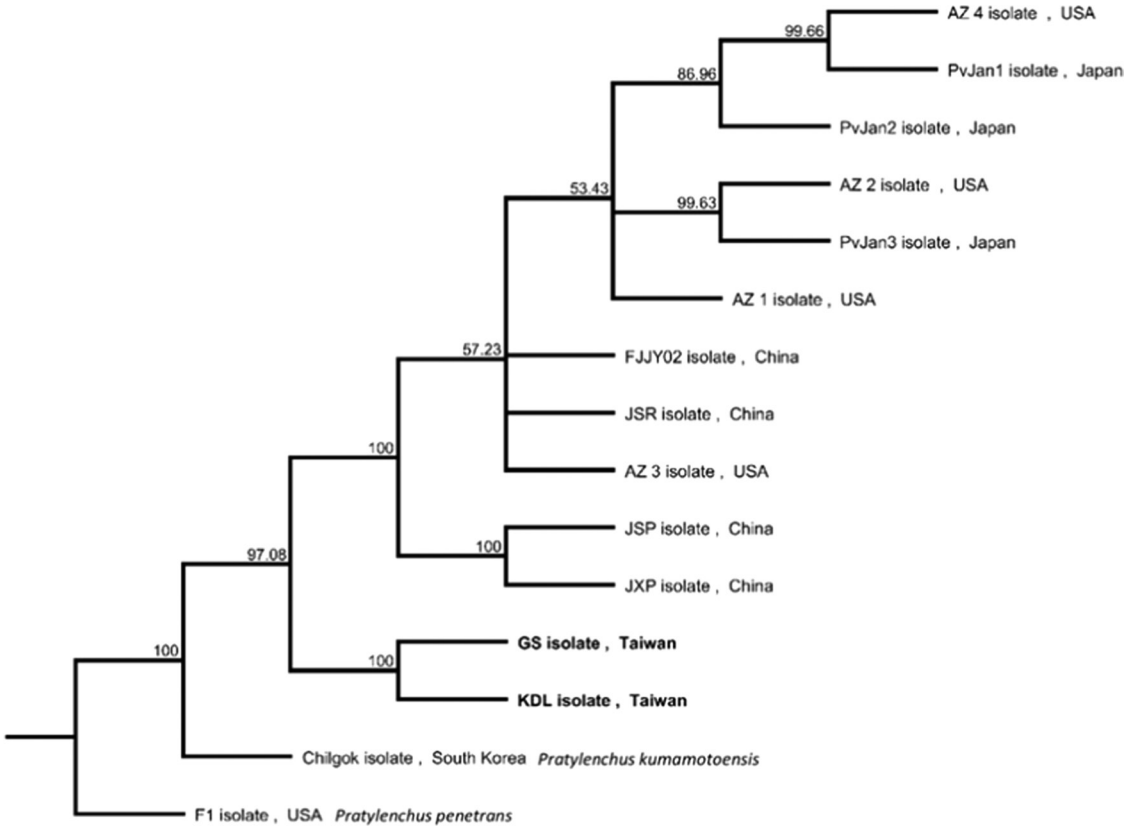

Figure 2: The phylogenetic tree of the combined partial 28S D2-D3 region and complete ITS region of the *P. vulnus* rDNA sequences. Morphologically similar species, *Pratylenchus penetrans* F1 isolate and of *P. kumamotoensis* Chilgok isolate, were used as out groups. Each node is marked with the isolate code, species and origin. The number at the fork represents the percentage of the bootstrap tested with 10,000 times for the indicating result.

Table 2. Measurements of the Taiwan *P. vulnus* population morphological characteristics.

	Female (*n* = 30)			Male (*n* = 30)		
Characteristics	**Mean**	**SD m.**	**Range**	**Mean**	**SD m.**	**Range**
L (µm)	704.01	74.85	573.46–839.91	587.02	46.83	492.76–678.1
K (µm)	25.97	4.06	17.76–36.18	18.47	1.47	15.49–21.50
Stylet (µm)	14.5	1.1	12.6–16.5	13.76	0.73	12.18–15.40
Tail (µm)	32.87	3.93	24.35–41.10	28.74	4.07	22.28–39.80
a (ratio)	27.47	3.15	22.74–34.30	31.85	2.53	26.83–36.62
b (ratio)	6.1	0.6	4.87–7.16	5.7	0.47	4.88–6.95
b' (ratio)	4.86	0.42	4.07–5.59	4.51	0.37	3.92–5.26
c (ratio)	21.57	2.38	17.83–27.46	20.71	2.68	13.51–23.99
c' (ratio)	2.29	0.31	1.59–2.86	2.29	0.38	1.77–3.83
V (%)	78.34	1.67	73.84–81.09	–	–	–
Spicules (µm)	–	–	–	16.88	1.89	12.06–20.15
DGO (µm)	3.55	0.32	3.00–4.42	3.41	0.33	2.77–4.31

Our discovery of the existence and population dynamics of *P. vulnus* in Taiwan implies a new threat to the 1,639 million NTD (ca. $52 million) strawberry industry. Currently, no nematicide is registered for root-lesion nematode suppression in strawberry. Related cultural and physical control options are currently undergoing evaluation by agricultural extension agencies to prevent serious damage.

Acknowledgments

The authors thank Dr. Zeng-Yei Hseu (Soil Survey and Contamination Remediation Laboratory, National Taiwan University) for the soil characterization assistance. The authors also thank Dr. Shiuh-Feng Shiao (Laboratory of Insect Systematics, National Taiwan University) and Ms. Shiang-Jiuun Chen (Department of Life Science, National Taiwan University) for SEM assistance. This research was funded by the Council of Agriculture, Executive Yuan, Taiwan, Grant No. 107AS-1.2.7-ST-aA. The authors declare no conflict of interest.

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